REMARKS

Status of the claims

Claims 22-24 and 31 are pending in the application. Claims 22-24 and 31 stand rejected. Claims 25-30 and 32-34 are withdrawn from consideration.

Claims 22 and 31 have been amended to require that the gene that encodes a fructosyltransferase enzyme is a vegetable gene. Support for these amendments is found in the specification, *inter alia*, at page 5, lines 1-13. No new matter has been added.

Claim Rejections under 35 U.S.C. § 112

Enablement

Claims 22-24 and 31 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in such a way as to enable one skilled in the relevant art, to make and/or use the invention. The Examiner stated that the claims are broadly drawn to a method for producing oligosaccharides having a low degree of polymerization, and a method for producing food products comprising selecting any gene coding for any fructosyltransferase (SFT) enzyme, linking the gene to suitable transcription-initiation and termination signals to provide an expression construct, and transforming any mutant species of plant cell with said expression construct. The Examiner stated that the specification only provides guidance for transforming *Nicotiana plumbaginifolia* with the full-length 6-SFT gene from barley. He also stated that it does not provide guidance for any mutant forms of *Nicotiana plumbaginifolia*, or any other species or any other mutant species transformed with any other fructosyltransferase genes resulting in oligosaccharides with a low degree of polymerization.

The Examiner asserted that the function of different 1-FFT genes is unpredictable. Vergauwen et al. (2003) (*Plant Physiol. 133*:391-401) was cited as providing a review of the literature on the role of 1-FFT in inulin production in different species, which noted different

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degrees of polymerization among different species of plants, particularly chicory and globe thistle. He also stated that not all 1-FFT genes have been characterized in all plant species, or in other species as broadly claimed in the present application, and undue experimentation, therefore, would be required to isolate, sequence, and characterize the enzymatic function of 1-FFT genes of all species.

The Examiner also stated that the specification does not provide any guidance for functional domains of 1-FFT or SST genes, or the amount of conservation in the DNA sequence that would be required for the activity of the enzymes encoded by the DNA sequences. Undue experimentation, therefore, would be required to determine all the embodiments of all 1-FFT and SST genes that are sufficient to express a protein capable of producing oligosaccharides having a low degree of polymerization.

Ritsema et al. (2004) (*Plant Molecular Biology 54*:853-863) was cited as evidence for the discovery that molecules with different fructosyltransferase activities can be created by mutagenesis and switching the sucrose binding boxes of FFT, SST, and invertase. Caimi et al. (WO 95/13389) was also cited as disclosing point mutations in the bacterial FTF gene, transformed into tomato plants resulted in full-length RNA transcripts that are not translated into functional FTF proteins. Undue experimentation, therefore, would be required to evaluate all such genes for their effect in all plant species, particularly those with phenotypes affecting starch or sucrose metabolism.

The Examiner also stated that the viability of transgenic plants that accumulate oligosaccharides is unpredictable. Turk et al. (1997) (*New Phytology 136*:29-38) was cited as disclosing transgenic tobacco plants transformed with *E. coli* levansucrase gene may have deleterious defects in the form of bleached leaves, stunted growth, and reduced root growth. Caimi et al. (supra) was cited as disclosing transgenic tobacco plants containing the bacterial

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fructosyltransferase gene SacB lack viability in their shoots. Undue experimentation, therefore, would be required to evaluate all fructosyltransferase coding sequences for their effect on oligosaccharide production and plant viability for all naturally occurring and molecularly-modified mutant plant species as broadly claimed.

Without acquiescing in the Examiner's conclusions with respect to enablement and written description, the applicants have amended Claims 22 and 31 to recite methods for producing oligosaccharides having a low degree of polymerization which include as their first two steps: (a) selecting a vegetable gene which ~~codes for~~ encodes a fructosyltransferase enzyme which converts sucrose into an oligosaccharide having a low degree of polymerization; and (b) linking the vegetable gene to suitable transcription-initiation and transcription-termination signals to provide an expression product. These amendments, we believe will facilitate prosecution, and simplify the response to 35 U.S.C. § 112 rejections cited above. The comments below attempt to address all of the other issues noted by the Examiner.

Vergauwen was cited for its review of the role of fructan:fructan 1-fructosyltransferase (1-FFT) in inulin production in different species, which noted different degrees of polymerization among different species of plants, in particular, chicory and globe thistle. Inulin, one of the simplest of the fructans, consists of α-Glc and a variable number of β-Fru moieties exclusively linked by 2-1 bonds to each other. Vergauwen notes that "Several plant 1-FFT enzymes have been purified and characterized" (lines 19-20, page 391). A general feature of these enzymes is the inability to use Suc as a donor substrate. A number of their cDNAs have also been cloned (citations omitted).

While Vergauwen states that analyzing the enzymatic mechanism of plant 1-FFTs is a challenging task, due to the complicating fact that both products of 1-FFT are also substrates (and vice versa), knowledge of the kinetic parameters and enzymatic mechanism, ping-pong or

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otherwise, is not terribly important or a prerequisite to taking steps to clone an enzyme, when sufficient protein is available for amino acid sequence determination used in the design and synthesis of DNA amplification primers. Vergauwen also notes (on page 396) that it is impossible to determine the exact kinetic parameters of the (four) reactions, including Reaction 4, which cannot be measured at all. These limitations, however, have not stopped researchers from cloning these enzymes, to facilitate study of their enzymatic mechanisms, compared to more applied research "with transgenic plants harboring different 1-FFTs showed that the properties of the 1-FFT enzymes involved are probably responsible for the degree of polymerization found" (citation omitted).

Knowledge, direct or indirect, of all the biochemical parameters, including active site moieties, and perhaps post-translational modifications which modulate enzyme activity in more subtle ways, is often not required or a barrier (illustrating the power of molecular biology) to carrying out procedures (which are now, and at the time the application was filed, were routine) designed to clone a desired enzyme. We also note that the pending claims are directed to methods, and not to specific molecules.

Ritsema was cited for the discovery that molecules with different fructosyltransferase activities can be created by mutagenesis and by switching the sucrose binding boxes of FFT, SST, and invertase. The applicants have noted that the senior author, and presumably principal investigator on this paper, is Sjef Smeekens, who is one of the inventors listed on the present application (as Josephus Christianus Maria Smeekens) which takes continuous priority back to a commonly owned foreign patent document filed on July 8, 1994. The same person is listed as Sjef C.M. Smeekens on several other published papers (not shown). The applicants have also noted that Ritsema was published in 2004 (received March 17, 2004, accepted in revised form on May 10, 2004) which post-dates the October 21, 2003 filing of the present application.

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The Examiner contends that the specification, as filed, does not provide any guidance for functional domains of 1-FFT or SST genes, or the amount of conservation in the DNA sequence that would be required for the activity of the enzymes encoded by the DNA sequences. It is the Examiner's position that undue experimentation would be required to determine all the embodiments of all 1-FFT and SST genes that are sufficient to express a protein capable of producing oligosaccharides having a low degree of polymerization.

The applicants respectfully disagree, however, since there is sufficient information supplied in the specification to support the claims as presented. Ritsema et al. states that fructosyltransferases as well as invertases belong to a family of 32 glycosyltransferases (page 854). A highly conserved sucrose binding box, is conserved in invertases, with a consensus sequence of HXXPXXX[LIVM]NDPN[GA] (dashes omitted), with the NDPNG/A pattern called the β-fructoidase motif. In fructosyltransferases, the sucrose binding box is also present, with some variations leading to a different consensus HXX[PTV]XXXX[LIVMA][NSCAYG][DE]P [NDSC][GA]. Ritsema et al. also states that "Sucrose binding boxes of enzymes originating from different plants, but encoding the same enzymatic specificity are often more homologous to each other than sucrose binding boxes of proteins originating from the same plant, but encoding different enzymatic activities (Figure 2 of Ritsema 2004, and citing Ritsema and Smeekens (2003)). They conclude by saying that "the region containing the sucrose-binding box is important in determining product type, but not substrate specificity."

Figure 2 of this reference shows a comparison of the β-fructosidase motifs of vacuolar invertases and fructosyltransferases. Figures 11 and 12 of the current specification also display the conserved amino acid sequences and a dendrogram showing their evolutionary relationship, respectively, of 6-SFT from barley with different invertases, levanases, and levansucrases. The

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dendrogram in Figure 12 was generated with the sequences presented in Figure 11 through the use of the Pileup Program of the UWGCG sequence analysis software package. These tables, the figures, and the text of the specification show that the applicants were in possession of skills and data which effectively demonstrated the ability to identify and predict with good confidence the conserved regions of proteins which possess or should possess fructosyltransferase activity.

The specification, as filed, also demonstrates that the applicants were also in possession of skills which demonstrate the ability to clone fructosyltransferases and related enzymes from various plant sources. The application discloses the use of the *ftf* gene of *Streptococcus mutans*, and the SacB gene of *Bacillus subtilis*, and several vegetable enzymes which are capable of converting sucrose into an oligosaccharide, such as the SST gene from onion, the 6-SFT gene from *Hordeum vulgare*, and the FFT gene from *Helianthus tuberosus*.

The sequence of cDNA encoding 6-SFT from barley is provided in Figure 8. The design of a probe is taught on page 25. The fragment was labeled with a random priming kit according to the instructions provided by its manufacturer. A cDNA library was prepared using a cDNA synthesis kit, and the library was screened with the labeled probe isolated from barley. The screening and isolation of positive clones were performed according to protocols supplied with the kit. The expression of the cDNA in *Nicotiana plumbaginifolia* protoplasts was described. Protoplasts were processed and the fructosyltransferase activity measured *in vitro* by established assays. The specification also teaches the construction of a vector and its transformation into plants (pages 1, 5, and Example 2).

These figures and their descriptions, coupled with the demonstration of methods for the cloning of 6-SFT from Barley (Example 5), SST from Onion (Example 3), and fructan-fructan fructosyltransferase from Jerusalem Artichoke (*Helianthus tuberosus* L.) (Example 6) are sufficient evidence that the applicants were in possession of sufficient information to determine

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the functional domains of 1-FFT or SST genes, or the amount of conservation in the DNA sequence that would be required for the activity of the enzymes encoded by the DNA sequences.

Finding other vegetable genes which are capable of converting sucrose into an oligosaccharide can be readily accomplished without undue experimentation by a skilled person in the art. The presence of an enzyme in a plant can be confirmed using a simple TLC assay (as described on page 9 of the present application). After confirming the presence of the enzyme in the plant, a skilled person would be able to isolate the gene of interest using well known techniques, including those related to protein purification, DNA sequencing, and molecular cloning.

The inventors also demonstrated the ability to transform the *ftf* gene from *Streptococcus mutans* described in Example 1 into different plants which included *Nicotiana* (Example 2), and into crop plants that included potato, beet (*Beta vulgaris* L), and *Brassica napus* (Example 4). The specification discloses other specific examples of plants that can be modified, including maize, wheat, barley, rice, soya bean, pea, bean, chicory, sugar cane, sweet potato, cassava, and grasses.

These results clearly demonstrate that the applicants were in possession of the claimed invention, having demonstrated methods within reach of the skilled person to isolate and characterize fructosyltransferases from plant sources, to design primers based on amino acid sequence information, to clone their genes, and to transform these genes into various crop plants and grasses. In view of the foregoing amendments and remarks, the applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph relating to enablement, be reconsidered and withdrawn.

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Written Description

Claims 22-24 and 31 were also rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time of filing, had possession of the claimed invention.

The Examiner stated that the claims are broadly drawn to a method for producing oligosaccharides having a low degree of polymerization, and a method for producing food products comprising selecting any gene coding for any fructosyltransferase (SFT) enzyme, linking the gene to suitable transcription-initiation and termination signals to provide an expression construct, and transforming any mutant species of plant cell with said expression construct. The Examiner also stated that the specification only provides guidance for transforming *Nicotiana plumbaginifolia* with the full-length 6-SFT gene from barley, and it does not provide guidance for any mutant forms of *Nicotiana plumbaginifolia*, or any other species or any other mutant species transformed with any other fructosyltransferase genes resulting in oligosaccharides with a low degree of polymerization. As a result of these propositions, the Examiner concluded that one skilled in the art would not have recognized that the Applicant was in possession of the claimed invention at the time of filing.

The Examiner did note, however, that the prior art does not teach or reasonably suggest methods for producing oligosaccharides having a low degree of polymerization (as claimed in Claims 21-23) or a method for producing food products (as claimed in Claim 31) comprising said oligosaccharides as a sugar substitute, comprising transforming a plant cell from a mutant plant having an altered starch or sucrose metabolism with an expression vector comprising a gene encoding a fructosyltransferase gene, regenerating a transgenic plant from the plant cell, culturing the transgenic plant, and isolating the oligosaccharides from the plant.

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Caimi et al. was cited as disclosing point mutations in the bacterial FTF gene, transformed into tomato plants resulted in full-length RNA transcripts that are not translated into functional FTF proteins. The Examiner asserted that undue experimentation would be required to evaluate all such genes for their effect in all plant species, particularly those with phenotypes affecting starch or sucrose metabolism.

Please note, as mentioned above, that the applicants have amended the claims to recite methods that use vegetable genes encoding a fructosyltransferase to facilitate the production of oligosaccharides.

It is also important to distinguish the methods and goals of Caimi et al. with those described by the inventors in the current application. Caimi et al. sought to describe methods for the synthesis and accumulation of fructose polymers in transgenic plants by selective expression of bacterial fructosyltransferase genes. Although they state that microbial fructans biosynthesis is understood well enough to allow the regulation of plant fructan accumulation through genetic engineering, indiscriminate expression of such genes could lead to altered concentrations of sucrose levels in various tissues "leading to unknown, but certainly serious negative results." It should also be noted that Caimi et al. sought to accumulate high molecular weight polymers, compared to the opposite goal of the instant application, which is accumulation of oligosaccharides having a low degree of polymerization. The high molecular weight polymers described in Caimi et al. are preferred as a bulk source of material, which are sequentially processed by enzymes into fructose-based sweeteners, such as corn syrup. The challenge in using microbial fructosyltransferase genes in plants, however, is the need to construct altered bacterial genes which contain plant targeting sequences that direct the heterologous enzyme into a desired organelle, such as a vacuole, or to the cytoplasm where it can function to modify oligosaccharide biosynthesis and degradation in a desired fashion. The microbial genes will

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almost certainly have altered codon preferences and AT content compared to functionally-similar plant genes, which may reduce the level of expression, by modulating mRNA levels and sizes and subsequent translation. In Example 8, Caimi et al. noted that the genetic lesion in its mutant sh4 line, which leads to increased sucrose levels, also affects pyridoxal phosphate metabolism. Lower transcript and protein levels were also observed in sh4 seeds, and although they had higher sucrose levels, higher fructan levels were not observed.

Turk et al. was cited as disclosing transgenic tobacco plants transformed with *E. coli* levansucrase gene may have deleterious defects in the form of bleached leaves, stunted growth, and reduced root growth. The applicants have also noted that the senior author on this paper is Sjef C. M. Smeekens, as described above. Turk et al. like Caimi et al., discloses transgenic plants harboring bacterial fructosyltransferase genes. In this study, however, the plant vacuolar targeting sequence of the sporamin protein from sweet potato was fused to a mutant GUS (β-glucuronidase) protein which no longer can be glycosylated, and separately to a C-terminal extended levansucrase protein from *Bacillus subtilis*, that was previously shown to synthesize fructan polymers of higher molecular weight than fructans synthesized by a levansucrase which lacks this extension. Bleaching of the leaves, stunted growth, reduced root growth together with an accumulation of glucose, fructose, sucrose, and starch was observed in the offspring of the highest expresser (SP-LS line 40). It should be noted, however, that the levansucrase was not properly translocated to the vacuole, suggesting that a retention signal present in the levansucrase protein might interfere with protein secretion in plants.

Both examples cited by the Examiner are related to plants transformed with bacterial levansucrases and fructosyltransferases. Neither reference discloses the use of *plant* fructosyltransferase genes in transgenic plants, that may not have undesired translocation effects observed with bacterial enzymes. In view of the amendments to Claims 22 and 31 to recite plant

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fructosyltransferases, the applicants respectfully request that rejections based on Caimi et al. and Turk et al. be reconsidered and withdrawn.

The Examiner also stated that the specification only provides guidance for transforming *Nicotiana plumbaginifolia* with the full-length 6-SFT gene from barley, and it does not provide guidance for any mutant forms of *N. plumbaginifolia*, or any other species or any other mutant species transformed with any other fructosyltransferase genes resulting in oligosaccharides with a low degree of polymerization.

The specification teaches how to prepare mutated forms of a fructosyltransferase gene in Section 2 of Example 1. As a demonstration, the *ftf* gene of *Streptococcus mutans* was mutated with nitrosoguanidine (MNNG) which induces point mutations (T→C and G→A). Other mutagenesis methods (site-directed or random) and genes which code for fructosyltransferase genes from other organisms can likewise be used to select a gene for a mutant oligosaccharide-producing organism. Mutagenesis techniques have been and are now commonly used to introduce alterations into a gene of interest. Changes can be detected by first selecting or screening for phenotypic alterations in an expressed gene, or by detecting structural changes directly in an amplified nucleic acid (DNA) following mutagenesis. Sonnewald and Willmitzer (1992) was also cited in Example 4, as describing examples of natural plant mutants and plants modified by means of molecular and genetic techniques which have altered starch and sucrose metabolism.

The specification also provides support for transforming suitable mutant plants with DNA constructs encoding desirable enzymes (see lines 6-14, page 19, of the application as filed). A skilled person will be able to transform mutant plants with constructs encoding the enzymes described in the application.

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In view of the foregoing amendments and remarks, the applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph relating to the written description, be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing amendments and comments, Claims 22-24 and 31 are believed to be in condition for allowance.

Entry of the foregoing amendments, reconsideration and withdrawal of the grounds for rejection of each of the claims, and prompt favorable action are requested. The Examiner may contact the applicants' representative at the number set forth below to discuss any issues that will facilitate prosecution of the application.

Respectfully submitted,

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